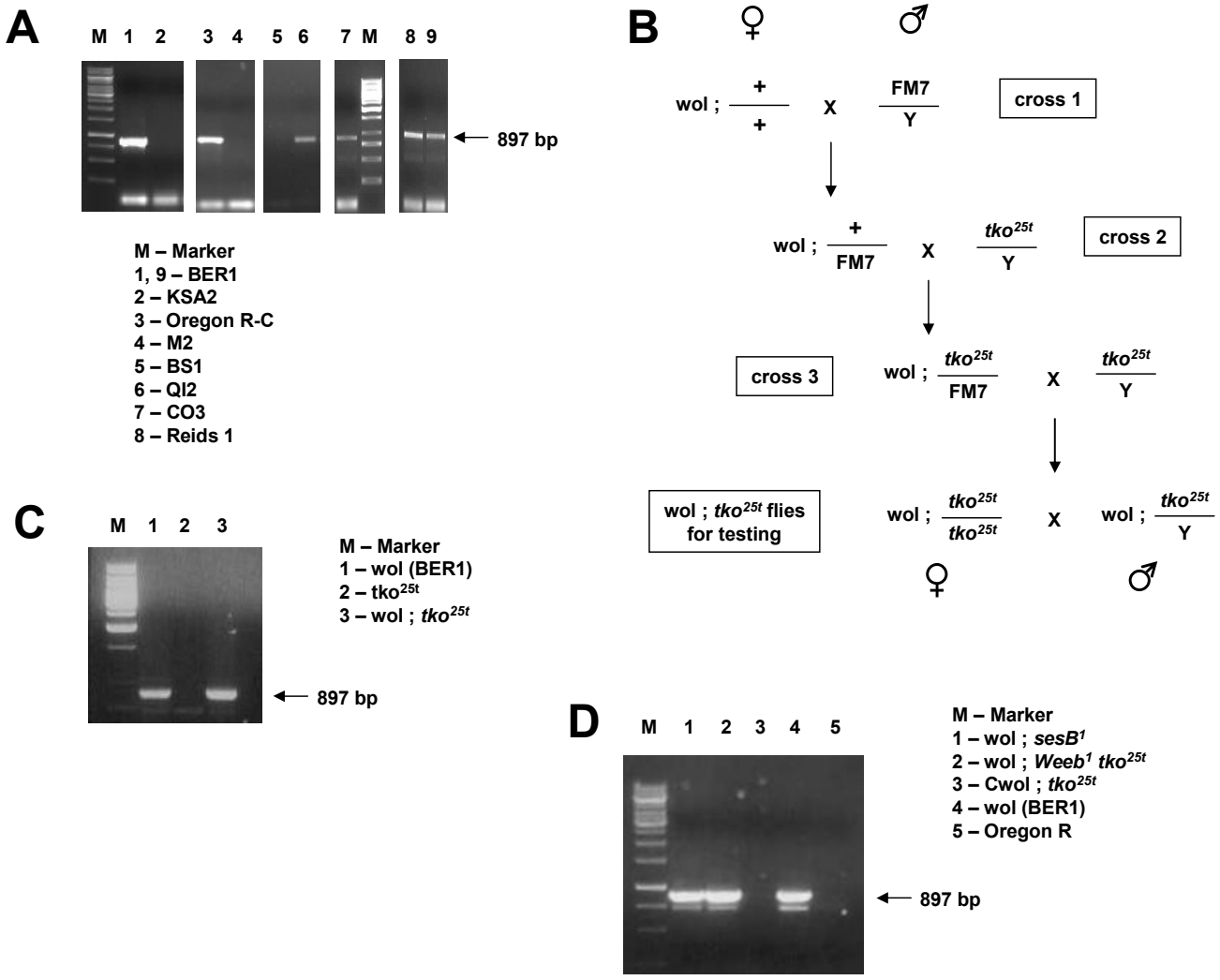
A
M 1 2 3 4 5 6 7 M 8 9
897 bp
M – Marker
1, 9 – BER1
2 – KSA2
3 – Oregon R-C
4 – M2
5 – BS1
6 – QI2
7 – CO3
8 – Reids 1
B
♀
♂
wol ; + / + X FM7 / Y
cross 1
wol ; + / FM7 X tko25t / Y
cross 2
cross 3
wol ; tko25t / FM7 X tko25t / Y
wol ; tko25t flies for testing
wol ; tko25t / tko25t X wol ; tko25t / Y
C
M 1 2 3
897 bp
M – Marker
1 – wol (BER1)
2 – tko25t
3 – wol ; tko25t
D
M 1 2 3 4 5
897 bp
M – Marker
1 – wol ; sesB1
2 – wol ; Weeb1 tko25t
3 – Cwol ; tko25t
4 – wol (BER1)
5 – Oregon R

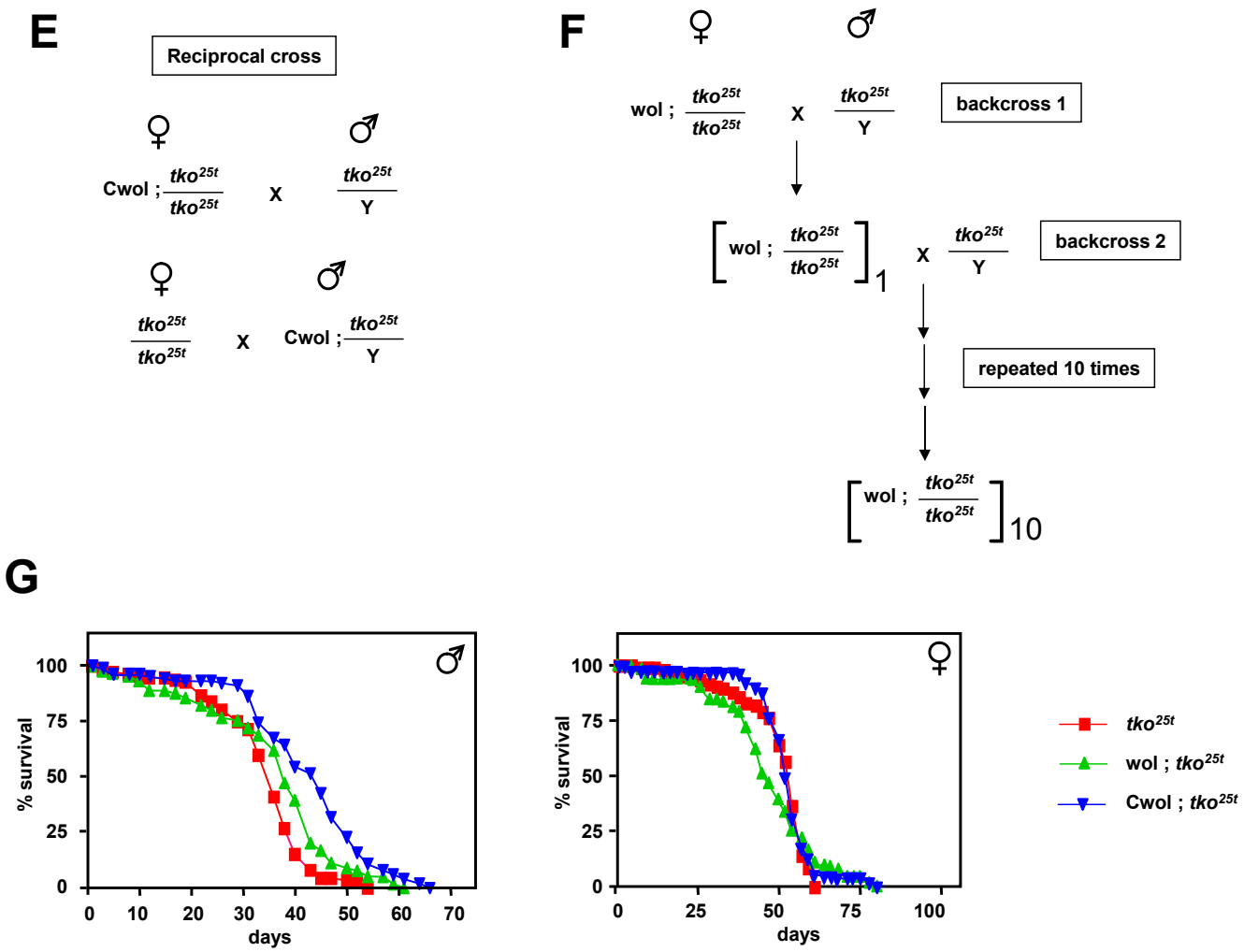

Figure S1 Creation and phenotypic characterization of cybrid *tko*^{25t} and *sesB*^{1} flies. (A) Representative gels of PCR products obtained using the *Wolbachia* 16S rRNA gene primers on genomic DNA of single individuals from the wild-type strains indicated. Gels run in parallel using the same marker ladder. Multiple individuals from these strains gave consistent findings. Infected strains BER1, Oregon R-C, QI2 and CO3 were maintained in quarantine and females were crossed into the *tko*^{25t} background using the crossing scheme shown in (B). Experimental crosses to test the effects of *Wolbachia* cytoplasms on the *tko*^{25t} phenotype used the *Wolbachia*-infected progeny from cross 3 (i.e. lacking the FM7 balancer), which were also maintained subsequently as both balanced and homozyous stocks. The presence of *Wolbachia* cytoplasm is denoted by wol. The use of the X-chromosome balancer excludes any suppressor effects that may be linked to *tko* itself, such as the segmental duplication studied previously (KEMPPAINEN et al. 2009). (C) The presence of *Wolbachia* from strain BER1 after introgressing its cytoplasm into the *tko*^{25t} nuclear background was confirmed by PCR, with template genomic DNAs as indicated. (D) *Wolbachia* was introduced into the *sesB*^{1} background by an identical strategy, and its presence confirmed by PCR, as shown. Its removal from the infected *tko*^{25t} line by tetracycline treatment (Cwol) was confirmed similarly, and by PCR with universal bacterial 16S rRNA gene primers (Table S2) which also gave no product. wol ; *Weeb*^{1} *tko*^{25t} – *Wolbachia*-infected *Weeble*^{1} *tko*^{25t} suppressor strain (KEMPPAINEN *et al*. 2009). (E) Reciprocal crossing scheme to test inheritance pattern of the suppressor phenotype of the BER1-derived *Wolbachia*-free Cwol ; *tko*^{25t} line. The demonstration of strictly maternal inheritance of the suppressor (Fig. 1D) rules out any significant contribution from nuclear DNA. (F) Backcrossing scheme to confirm the cytoplasmic inheritance of *tko*^{25t} suppression in the *Wolbachia*-infected strain BER1 (denoted wol). Note that this back-crossing scheme was also used to test other cytoplasms (Fig. 4), producing identical results for four different suppressors, again consistent with the suppressor determinant being purely cytoplasmic. (G) Lifespan curves for flies of the genotypes indicated. The curves for *Wolbachia*-infected (wol) and cured (Cwol) *tko*^{25t} males in the presence of BER1 mtDNA, after backcrossing for 10 generations, were significantly different (median survival of 43 d) from *tko*^{25t} males in the original (Oregon R-related) mtDNA background (median lifespan 31 d), $p < 0.0001$, log rank test). Because the flies are backcrossed to the same nuclear background, the differences are strictly attributable to the cytoplasmic genotype. Note that *tko*^{25t} flies have a much shorter lifespan than wild-type Oregon R flies tested in our laboratory (SANZ *et al*. 2010a).